Two-component transposon system for transgenic tomato

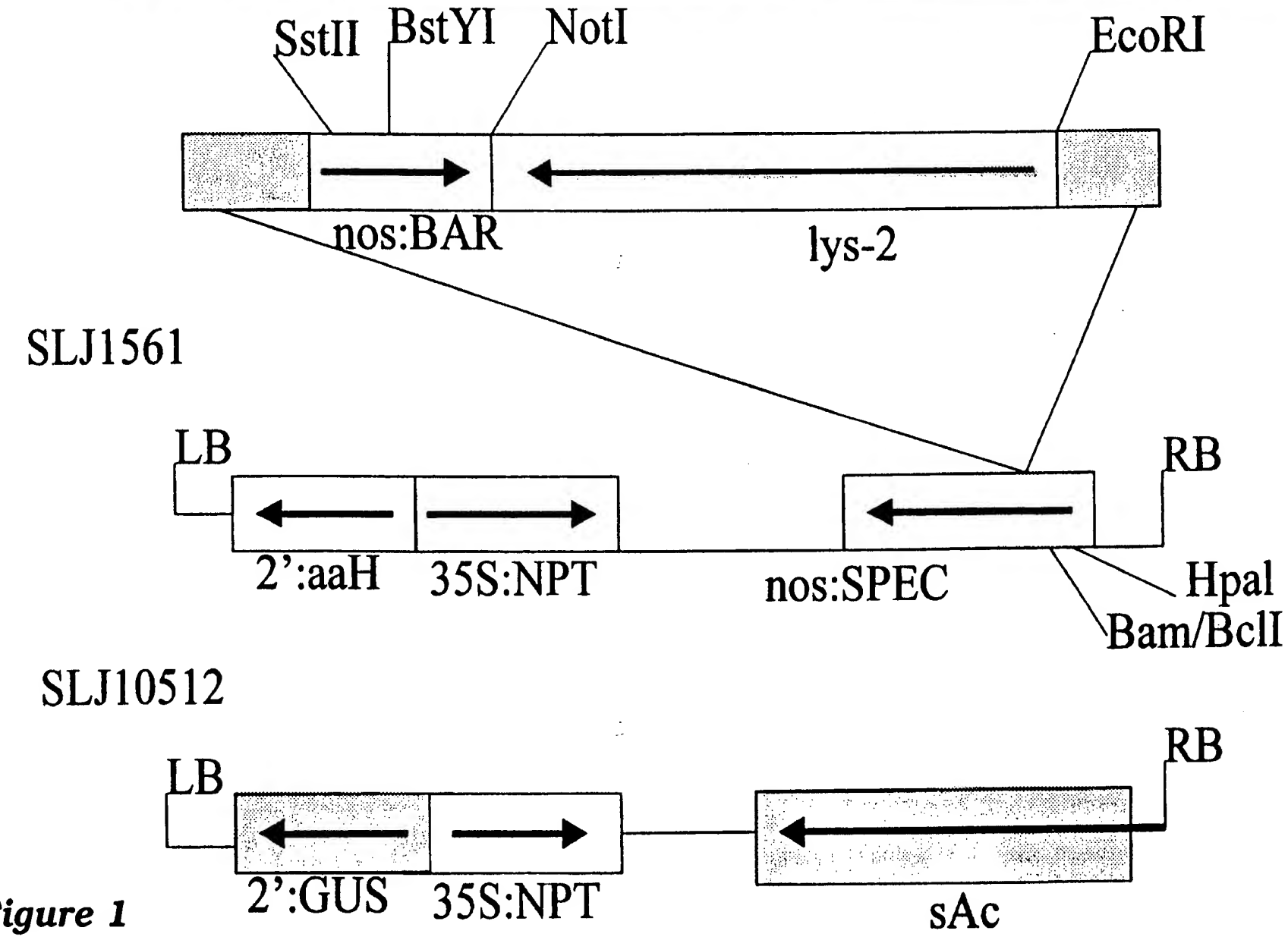

Figure 1

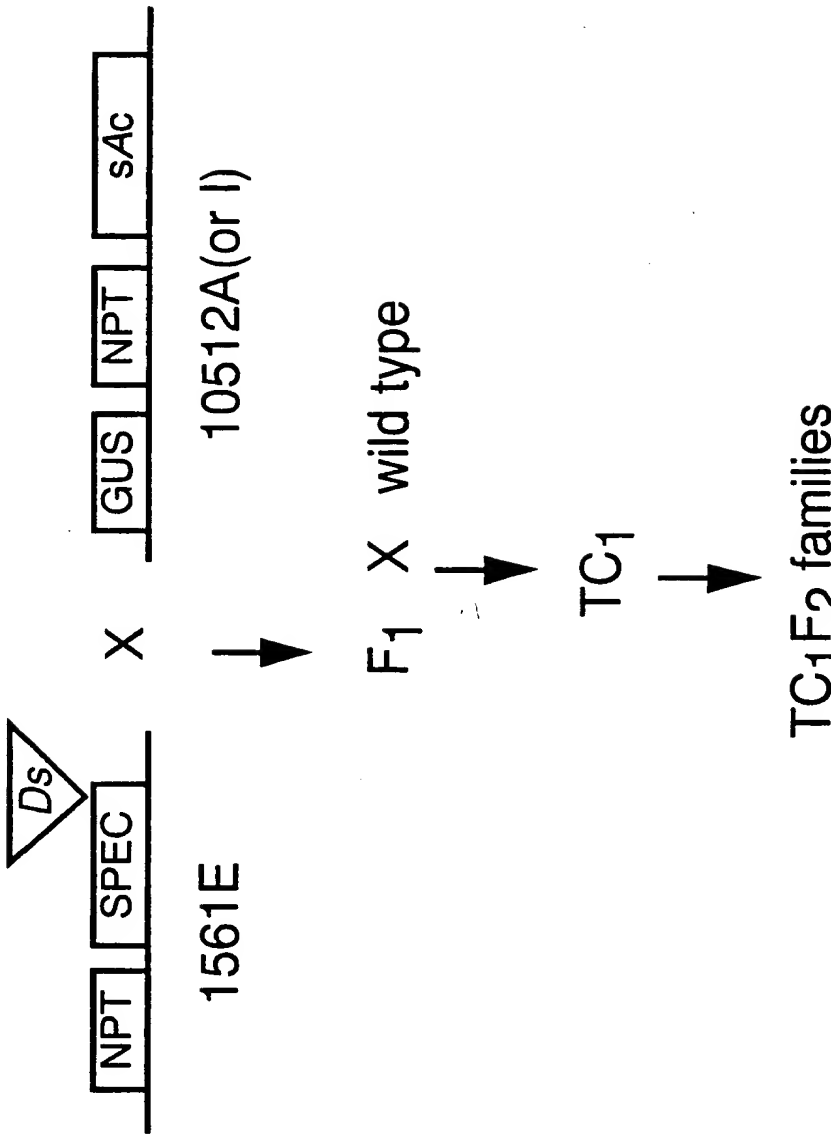
Ds
NPT
SPEC
1561E
X
GUS
NPT
sAc
10512A(or I)
F1 X wild type
TC1
TC1F2 families

Figure 2

SLJ1561 Ds element
BAR probe
Ss
Bs
Nt
lys-2 probe
R1
3'
5'
nos:BAR
lys-2
1 2 3 4 5 6 7
1 2 3 4 5 6 7
Sst II + BstYI digest, BAR probe
Not I + EcoRI digest, lys-2 probe

Figure 3

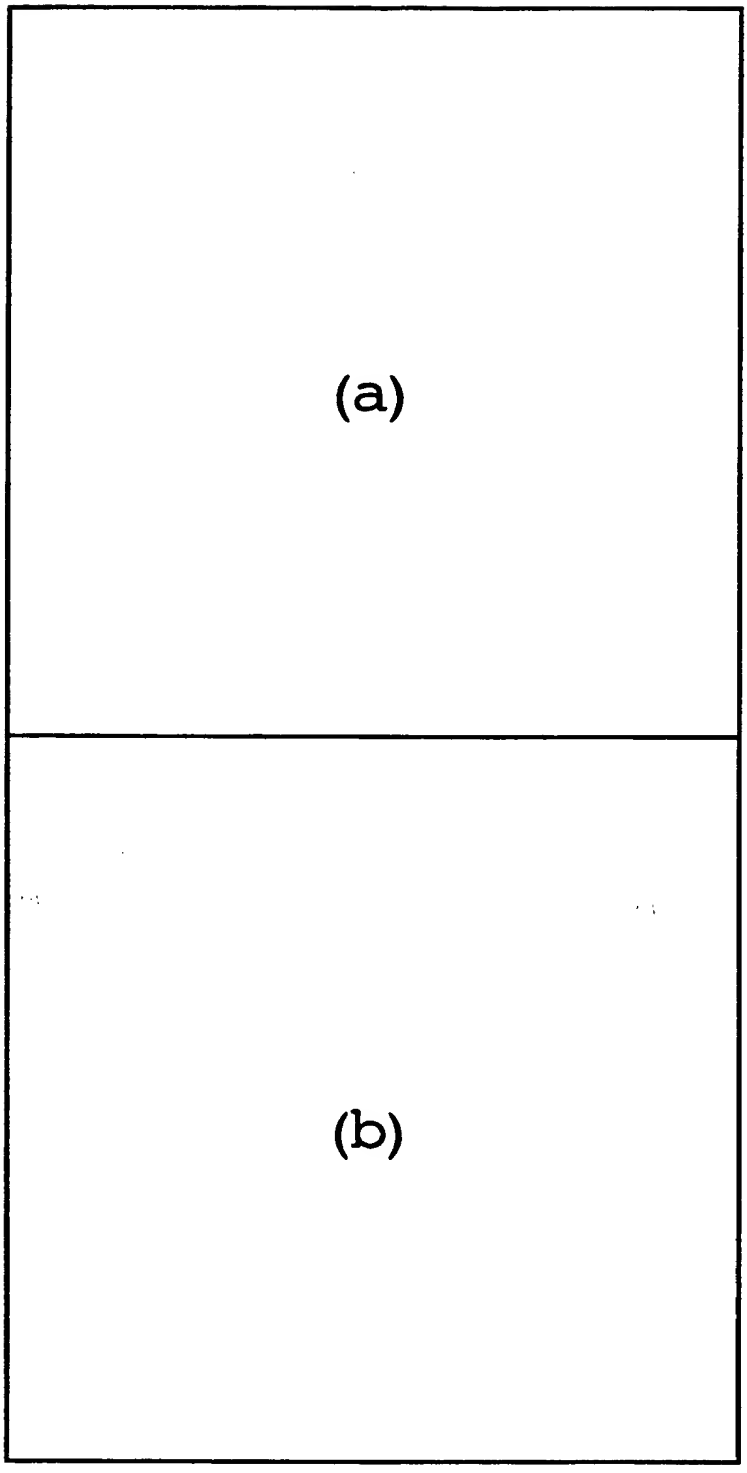

Figure 4(i)

FIGURE 4(i)

```
 981 TTTGAAATTTATGTATATATCTGTAGCATTAGAAACTATAAGAGTTGTTA 1030   Potato
     |||||||||||||||||| ||||| ||||||||||||||||||||||||||
  40 TTTGAAATTTATGTATTTATCTATAGCATTAGAAACTATAAGAGTTGTTA 89     Tomato

1031 GCTTCACTTGTCTTATTGTTGTGCTCAAAGCAACT...TCATCATACAGT 1077
     |||||||||| |||| ||||||||||||||||||   ||||||||||||
  90 GCTTCACTTGGCTTACTGTTGTGCTCAAAGCAACTTCATCATCATACAGT 139

1078 ATGGTTTTTATATGCTCTTCCATTATCACCGAACCTTATGATTATG.TGT 1126
     |||||||| |||||||||||||||||||| || |||||||||||| | |
 140 ATGGTTTTGATATGCTCTTCCATTATCACTGAGCCTTATGATTATGTTTT 189

1127 ACGAGCTTATAATATTACTGATGGTGATTCAGTATTATGATTATGTCCTC 1176
     |||||||||||||||| |||||||||||||||||||| ||||||||||||
 190 ACGAGCTTATAATATCACTGATGGTGATTCAGTATTGTGATTATGTCCTT 239

1177 CATTAATTATTCTGTTTCATACAAGTCGTGTAATTTGCTGTTTGTGATTG 1226
     | || ||||||||||||||||||||||||||||||||||||||||||  |
 240 CGTTGATTATTCTGTTTCATACAAGTCGTGTAATTTGCTGTTTGTGACAG 289

1227 TACGATAAATTGATTCAACCTTCTGCGGTGTTGGTTGAAGTTCAAGTAAA 1276
     ||||||| || || |||||||||||| ||| || |||||||||||| |||||
 290 TACGATAGATCGACTCAACCTTCTGAGGTATTAGTTGAAGTTCATGTAAA 339

1277 TTAGCTTTATTTATCATAGTAGCATTTGATTATTGATGCTCTGTAGCTAA 1326
     |||||||| ||||||||||||||||||||||||||||||||||||||||
 340 TTAGCTTTGTTTATCATAGTAGCATTTGATTATTGATGCTCTGTAGCTAA 389
```


Figure 4(i)(a)

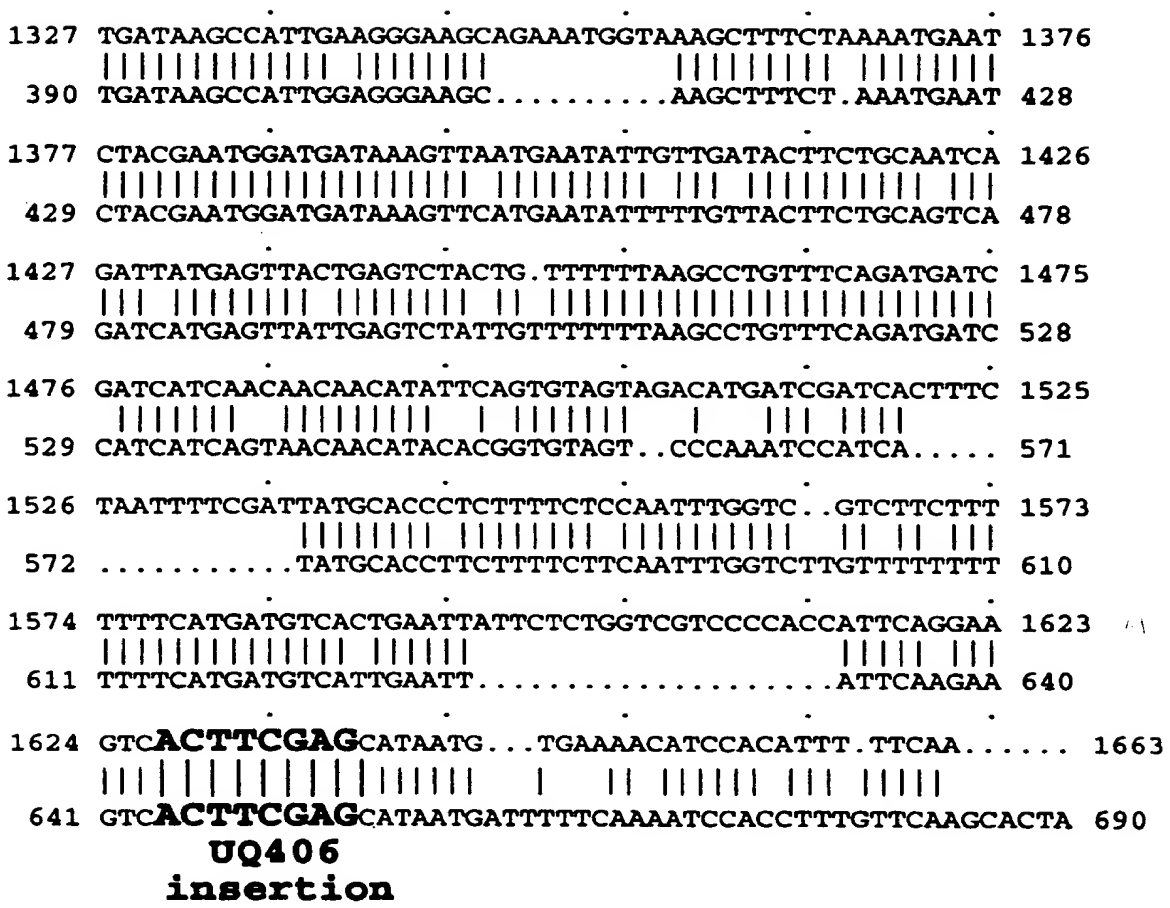

Figure 4(i)(b)

Substitute Sheet
(Rule 26) RO/AU

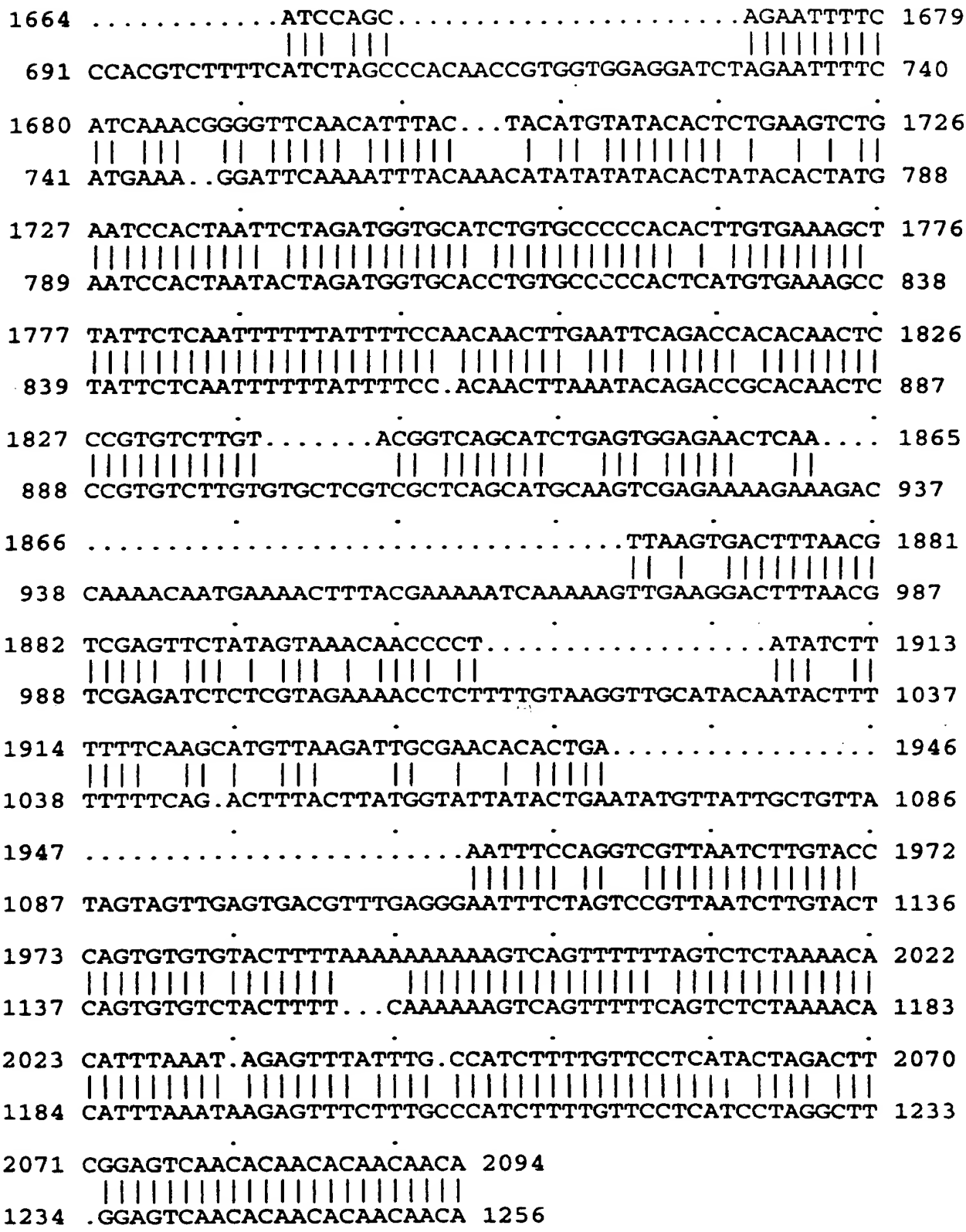

Figure 4(ii)

```
cgacggcccg ggctggtaaa tgcggaagct tgttacagat ttgaaattta      50
tgtatttatc tatagcatta gaaactataa gagttgttag cttcacttgg     100
cttactgttg tgctcaaagc aacttcatca tcatacagta tggttttgat     150
atgctcttcc attatcactg agccttatga ttatgttttta cgagcttata    200
atatcactga tggtgattca gtattgtgat tatgtccttc gttgattatt     250
ctgtttcata caagtcgtgt aatttgctgt ttgtgacagt acgatagatc     300
gactcaacct tctgaggtat tagttgaagt tcatgtaaat tagctttgtt     350
tatcatagta gcatttgatt attgatgctc tgtagctaat gataagccat     400
tggagggaag caagctttct aaatgaatct acgaatggat gataaagttc     450
atgaatattt ttgttacttc tgcagtcaga tcatgagtta ttgagtctat     500
tgttttttta agcctgtttc agatgatcca tcatcagtaa caacatacac     550
ggtgtagtcc caaatccatc atatgcacct tctttttctc aatttggtct     600
tgttttttt ttttcatgat gtcattgaat tattcaagaa gtcacttcga     650
gcataatgat ttttcaaaat ccacctttgt tcaagcacta ccacgtcttt     700
tcatctagcc cacaaccgtg gtggaggatc tagaattttc atgaaaggat     750
```

Figure 5(i)

```
tcaaaatttta caaacatata tatacactat acactatgaa tccactaata   800
ctagatggtg cacctgtgcc cccactcatg tgaaagccta ttctcaattt   850
tttattttcc acaacttaaa tacagaccgc acaactcccg tgtcttgtgt   900
gctcgtcgct cagcatgcaa gtcgagaaaa gaaagaccaa aacaatgaaa   950
actttacgaa aaatcaaaaa gttgaaggac tttaacgtcg agatctctcg  1000
tagaaaacct cttttgtaag gttgcataca atacttttct ttcagacttt  1050
acttatggta ttatactgaa tatgttattg ctgttatagt agttgagtga  1100
cgtttgaggg aatttctagt ccgttaatct tgtactcagt gtgtctactt  1150
ttcaaaaaag tcagttttc agtctctaaa acacatttaa ataagagttt   1200
ctttgcccat cttttgttcc tcatcctagg cttggagtca acacaacaca  1250
acaacaatga atttccattt ttctgtttct ttacttctct ctttatctct  1300
tcctatgttt gcctcttcga cggtgttatt tcaggtatcc atctccaaag  1350
aaccttattt ttctcttaac ttttcctatg tatatgtatc tctatgttta  1400
tgtagtactt gctcaagtat ataaaagaaaa gttagtttct ctagaatctt  1450
tgaattcatt tgttaggggt tcaattggga ttcgagtaat aagcaaggcg  1500
```

Figure 5(ii)

```
gatggtacaa ctctctcatc aacttagttc cggacttggc taaagctgga 1550
gttactcatg tttggttgcc accatcatct cactccgttt ctcctcaagg 1600
taattttcgg agtgattgtg acctagtaat ccaatgaagt caaaataacc 1650
acggaagatt agagtctaaa ttttaatgaa aatagttcag acaagttaat 1700
gaccaactta tatattagtt caatccataa aatttgatgt agtagttaca 1750
aaatggaatt gcttgaaggc ttatgccatg ttttatgcca ggttatatgc 1800
caggaaggtt gtatgactag gatgcttcca agttttggaaa tcagcaacaa 1850
ctgaaaactc ttattaaggc tttaacatga ccacgggatc aaatcggttg 1900
ctgatatagt gataaatcat agaactgctg ataacaaaga tagcagggga 1950
atatacagca tctttgaagg aggaacatct gatgaccggc ttgattgggg 2000
tccatctttc atttgcagga acgacacaca atattctgat ggcacgggga 2050
atccagacac gggtttggac tttgaacctg cacctgatat cgatcatctt 2100
aatacgagag tgcagaaaga gttatcagac tggatgaact ggctgaaatc 2150
tgaaattgga tttgatggtt ggcgtttcga ttttgttagg ggatatgcac 2200
cttgcattac caaaatttat atgggaaaca cgtccccgga ttttgctgtt 2250
```

Figure 5(iii)

```
ggtgaattgt ggaactctct tgcttatggc caggacggga aaccggaata    2300
taaccaggac aatcatagaa atgagctagt tggttgggta aaaaatgcgg    2350
ggcgggctgt aacagctttt gattttacaa caaagggaat tcttcaagct    2400
gcagttcaag aagagttatg gagattgaag gatcccaatg gaaaacctcc    2450
tgggatgatc ggtgttttgc ctcgaaaagc tgtgactttt atcgataatc    2500
atgatactgg atcgacacaa aatatgtggc ctttcccttc agacaaagtt    2550
atgcaaggat atgcatacat tcttactcat ccaggaatcc catccgtggt    2600
aaaaaaaata aataaattct ttctacatat ctcattgttt tctattttac    2650
aagaaattta tattcttttc caggggattt gagaaactcg gcctgtggga    2700
gtttgctcac attgccagtc tcgtaatcca taaacaaaca ctcaaactct    2750
gagtgtgcac atctagacac ctcaactcgt ttttcaccgt gttaattgaa    2800
cacttcaact tacaaaatga tcgtgtagca cctccaaaaa ttatgtgtca    2850
caattagcca cgtgcgagat acacgaaaat gagttggagt agttagttgc    2900
caaataaaac caagctgagg tgtctaaatg tgcacnctca aagtnggatg    2950
tttacttggc agctgaggcc gaggccatgt ttgantgtta tgcttatagg    3000
```

Figure 5(iv)

atatgacaca tttgtttccg attagctgag ganttgatta aatcctngtt	3050
ttngttngca gtttnatnac cattnctttg atnggggctn cnaggatgga	3100
attncagcac taanctctat taggaaaagg aataggattt gtgcancaag	3150
caatgtgcaa ataatggctc ctgattctga atctttatat ancaatggat	3200
catcacaaaa tcattgtcaa gattggacca aaacttgatc ttggaaatct	3250
tattccacct aattatgagg tggcaacttc tggacaagac tatgctgtat	3300
gggagcaaaa ggcataatca tattgtacca cactaaaagg gaccatggcc	3350
acaatggttc tcattagtgt taatgttata tgattgaaaa tgtaatttat	3400
attgacataa tgaaggccaa aaattcaaga aattataaac aattcaatag	3450
tccttgctca attcacaatt acattatgac ttctctattg caaactagtt	3500
tgggtccaca ttattgtctc ctaaaatttt acaacatttc ttaagggaac	3550
ttaattagtt acagtgaaca tatgttgaaa ttacccttta tcccctttaca	3600
attgatttaa taaatatttc ccctatccct ttggtagttg gttagagtta	3650
taagtaacgt agagattagt tataagagaa tttatgtatt attatgcaga	3700
tgtttagtta tatcgatttt agttatttat atgttgatta tttcaccttc	3750

Figure 5(v)

```
aataatgcat ataaagatgg taaatgattg gattgatcga attcgaatga   3800
gtttgaatat gaactaatct tcaaatttaa tataaatttt ttttgtcaac   3850
atctatagcc aaacggctcc aaaacaataa ataatttaca tttattgtag   3900
tattttattt aaaatgggat nttcctcatc ccacttgtac cagttgaaac   3950
cctaataata agccaatcca accgtcaaaa ttacaaattt tgaaaattgc   4000
gctcctcaca gttctccccct attcagattt gattcatttct cttcattttt   4050
tgttttcaca ttttacctct aaatcaactc gagtcccttt gttcaaatgg   4100
gtgctaatca cagccgtgaa gatctggagc tttctgattc cgagtctgaa   4150
tccgaatatg ggtccgagtc tcgaacaaagg gaggaagagg aagacgaaga   4200
taactactca gatgctaaaa cgacgccgtc ttccactgat cggaaacaga   4250
gcaaaaccccc gtcttctttg gatgatgttg aagcaaagct gaaagcttta   4300
aagcttaagt atggtactcc tcatgctaaa acccccacag cgaaaaacgc   4350
tgttaaactt taccttcatg ttggtgggaa cactgcgaat tccaaatggg   4400
tagtttctga taaggtgaca gctttattcgt ttgttaaatc gggtagtgag   4450
gatggatcgg atgatgatga aaatgaagaa actgaggaga atgcttggtg   4500
```

Figure 5(vi)

```
ggtttttgaaa attgggtcga aggttcgggc taagattgat gagaatttgc   4550
agctcaaggc atttaaggag cagaaaaggg tggatttttgt ggcgaatggg   4600
gtttgggctg tgagattctt tgggaggaaa gagtataaag cgttcattga   4650
cttatatcag agctgtttgt ttgagaatac ttatgggttt gaggcaaatg   4700
atgagaatag agttaaggtg tatggtaaag actttatggg gtgggcaaat   4750
ccagaagctg cggatgattc aatgtgggag gatgctgggg atagcttcgc   4800
gaagagccct gcgtctgaaa agaagacacc tttgagggtt aaccatgatt   4850
tgagggagga gtttgaggag gcagctaaag gaggagctat tcagagcttg   4900
gcattaggtg cgttggataa tagttttctt ataagtgatt ctggaattca   4950
ggttgtgagg aactatactc atggaataag tggaaaaggt gtttgtgtca   5000
atttgataa ggaaaggtct gctgtaccta attccactcc aaggaaagct   5050
ctacttctaa gagctgagac taatatgctt ctcatgagtc cagtgactga   5100
tagaaagcct cactctcggg gattacatca gtttgatatc gagactggga   5150
aggttgttag cgagtggaag tttgagaaag atggaactga tatcacgatg   5200
agggatatca ctaatgatag caaaggagct cagatggatc cttcgggtc   5250
```

Figure 5(vii)

tactttctta gggctagatg ataacagatt gtgtaggtgg gatatgcgtg 5300
atcggcatgg gatggtccag aatctagttg atgaaagtac tcctgtgctg 5350
aattggactc aaggacatca attttcgagg ggaactaact ttcagtgctt 5400
tgctactact ggtgatggat caattgttgt tggttcactt gatggcaaga 5450
ttagattgta ctcaagcagt tccatgagac aggctaaaac tgcttttcca 5500
ggccttggtt ctcctatcac tcatgtggat gttacctatg atgggaagtg 5550
gatattgggg acaactgata cttacttgat attgatatgc accttgttta 5600
tcgacaagaa tggaactact aagactggtt ttgctggtcg catgggaaat 5650
aagatttccg ctccaagatt gttaaagcta aaccctctcg attcacatat 5700
ggctggagct aacaagttcc gcagtgctca attttcatgg gtcaccgaga 5750
atgggaagca agagcgccac ctcgttgcta ctgttgggaa gtttagtgtg 5800
atctggaatt ttcaacaggt gaaggatggt tctcatgagt gttaccagaa 5850
tcaggttggg ttgaagagct gctattgtta caagatagtc ctaagagacg 5900
actctattgt agaaagtcgt ttcatgcatg acaagtacgc tgtttctgac 5950
tcacctgaag caccactggc ggtagcaacc cccatgaaag tcagctcatt 6000

Figure 5(viii)

cagcatctct agcaggcgct tacaaatttg aacaatcatt ctgttcatat 6050
acgcaactta ttagatttat ctgtagcaga attagtgtct ctcacactaa 6100
gtagcttgaa aaactgcaca tctgcaaatc atttccagtt caatgtatta 6150
ctactttagt ttaaaaacct taaaaggcag tcttccaaat tctaggtatc 6200
ctcacctgac attattattg ttgtaatagc taattgttgc ttgctctaaa 6250
tccccgttca atg 6263

Figure 5(ix)

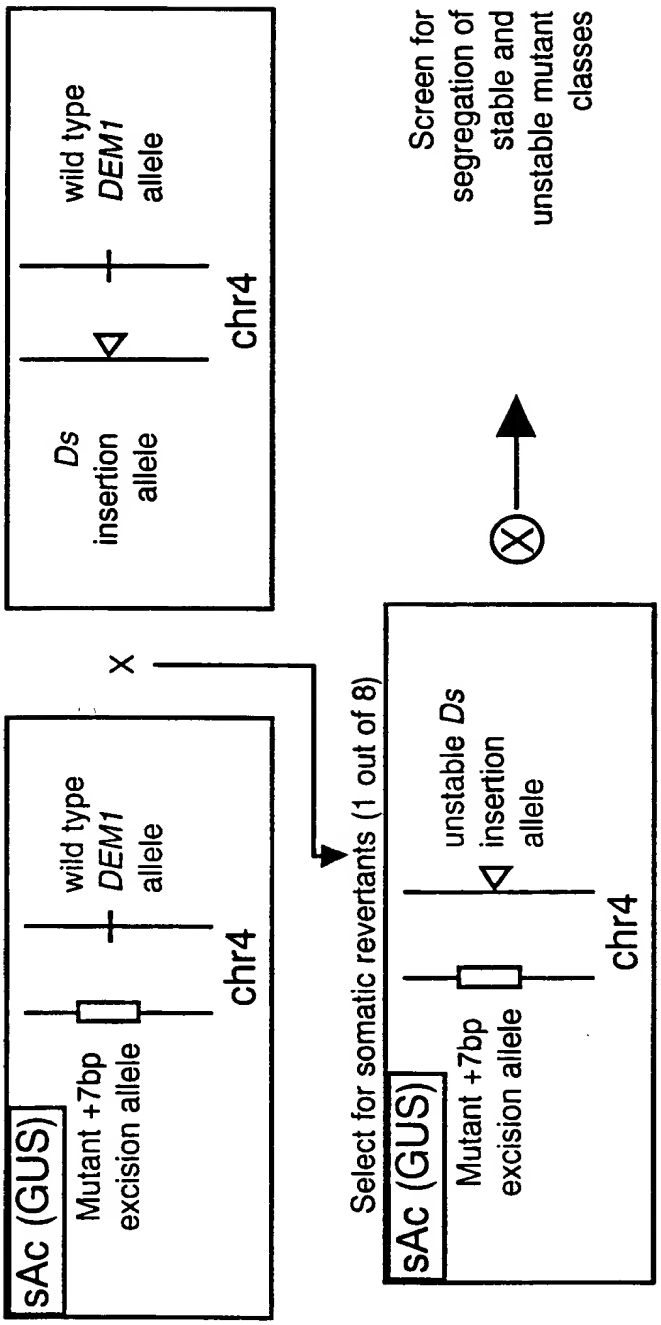
wild type
DEM1
allele
Ds
insertion
allele
chr4
Screen for
segregation of
stable and
unstable mutant
classes
X
sAc (GUS)
Mutant +7bp
excision allele
wild type
DEM1
allele
chr4
Select for somatic revertants (1 out of 8)
sAc (GUS)
Mutant +7bp
excision allele
unstable Ds
insertion
allele
chr4

Figure 6

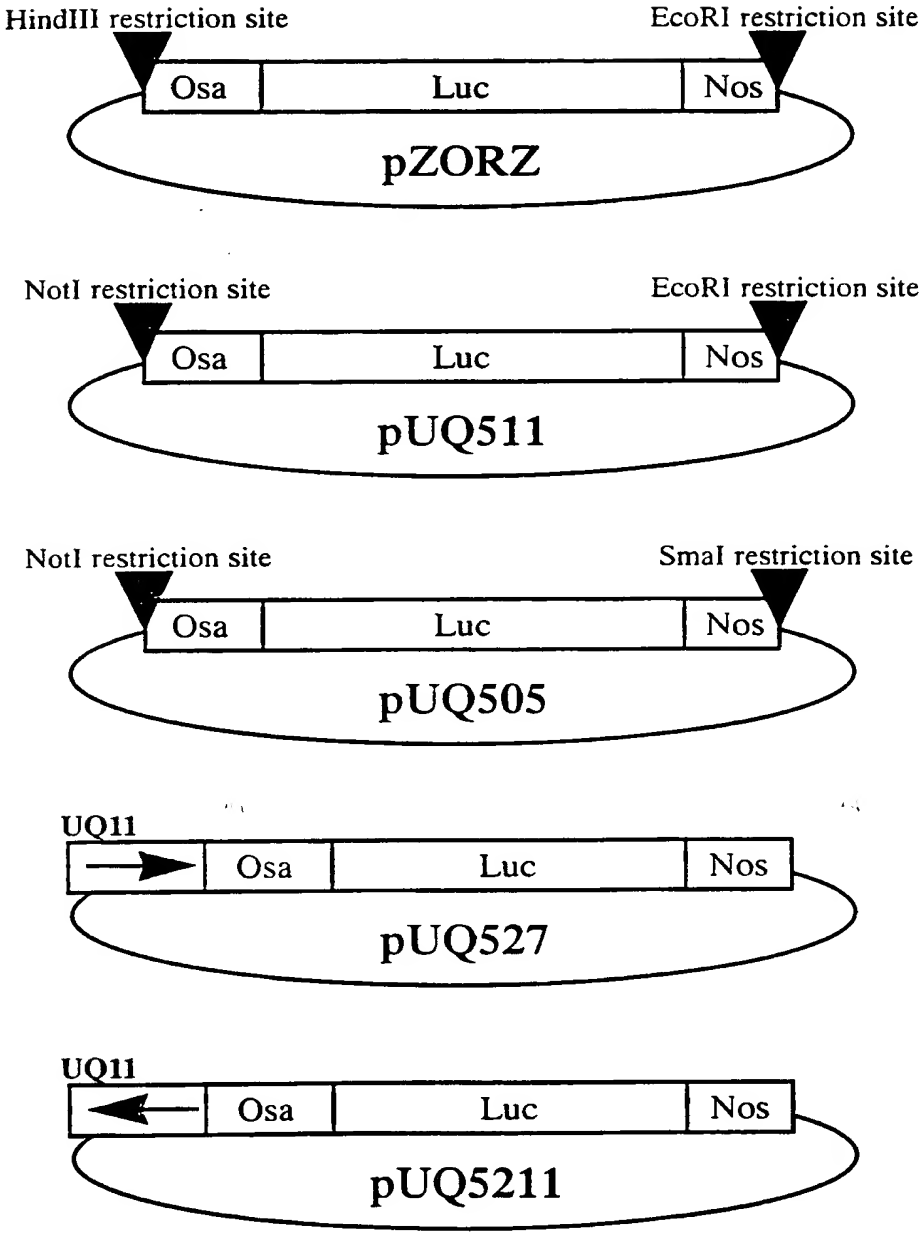

Figure 7

Substitute Sheet
(Rule 26) RO/AU

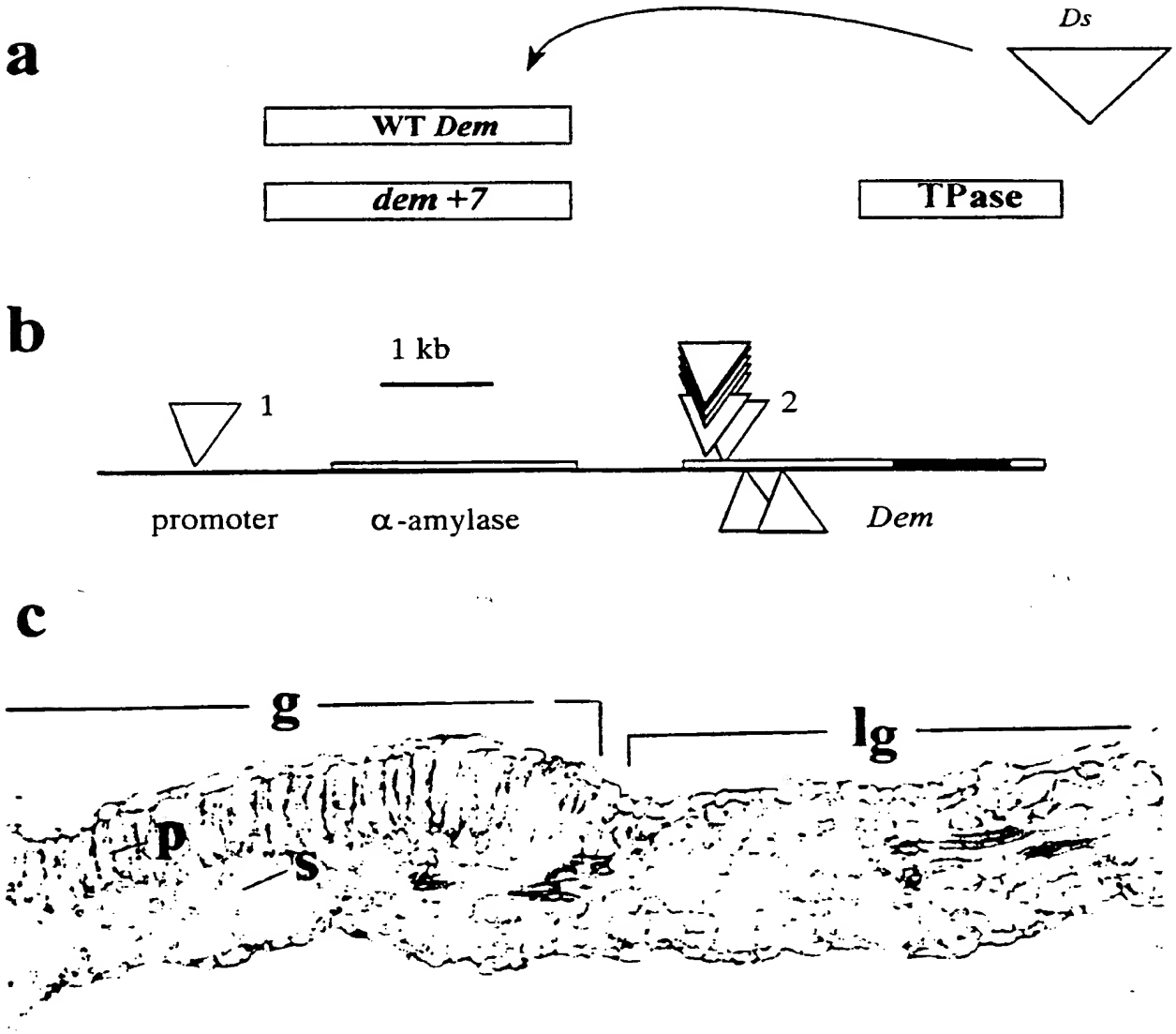

Figure 8

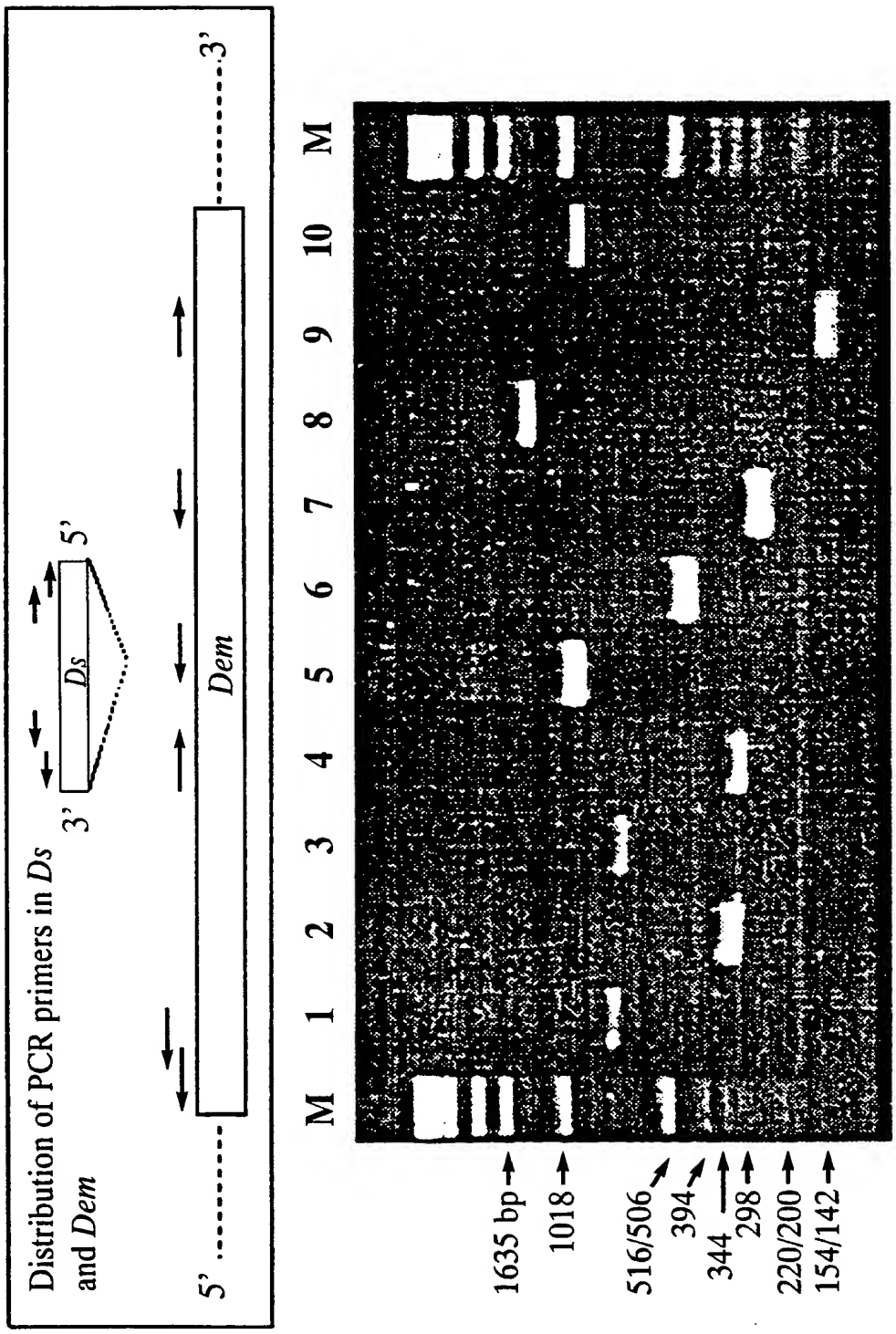
Distribution of PCR primers in Ds and Dem
5'
3'
Ds
3'
5'
Dem
5'
3'
M 1 2 3 4 5 6 7 8 9 10 M
1635 bp
1018
516/506
394
344
298
220/200
154/142

Figure 9

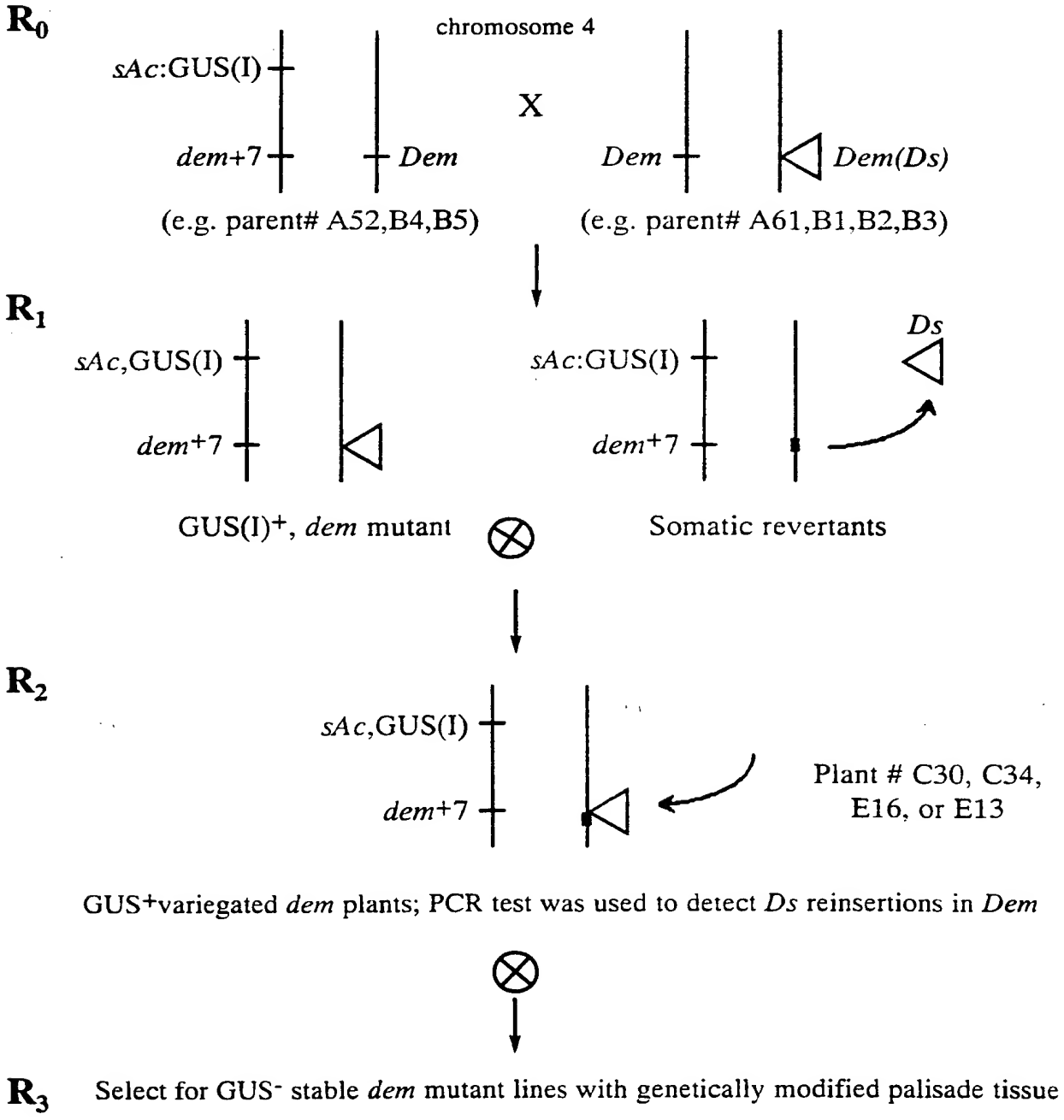
R0
chromosome 4
sAc:GUS(I)
dem+7
Dem
X
Dem
Dem(Ds)
(e.g. parent# A52,B4,B5)
(e.g. parent# A61,B1,B2,B3)
R1
sAc,GUS(I)
dem+7
sAc:GUS(I)
dem+7
Ds
GUS(I)+, dem mutant
Somatic revertants
R2
sAc,GUS(I)
dem+7
Plant # C30, C34,
E16, or E13
GUS+variegated dem plants; PCR test was used to detect Ds reinsertions in Dem
R3
Select for GUS- stable dem mutant lines with genetically modified palisade tissue

Figure 10

09/701926

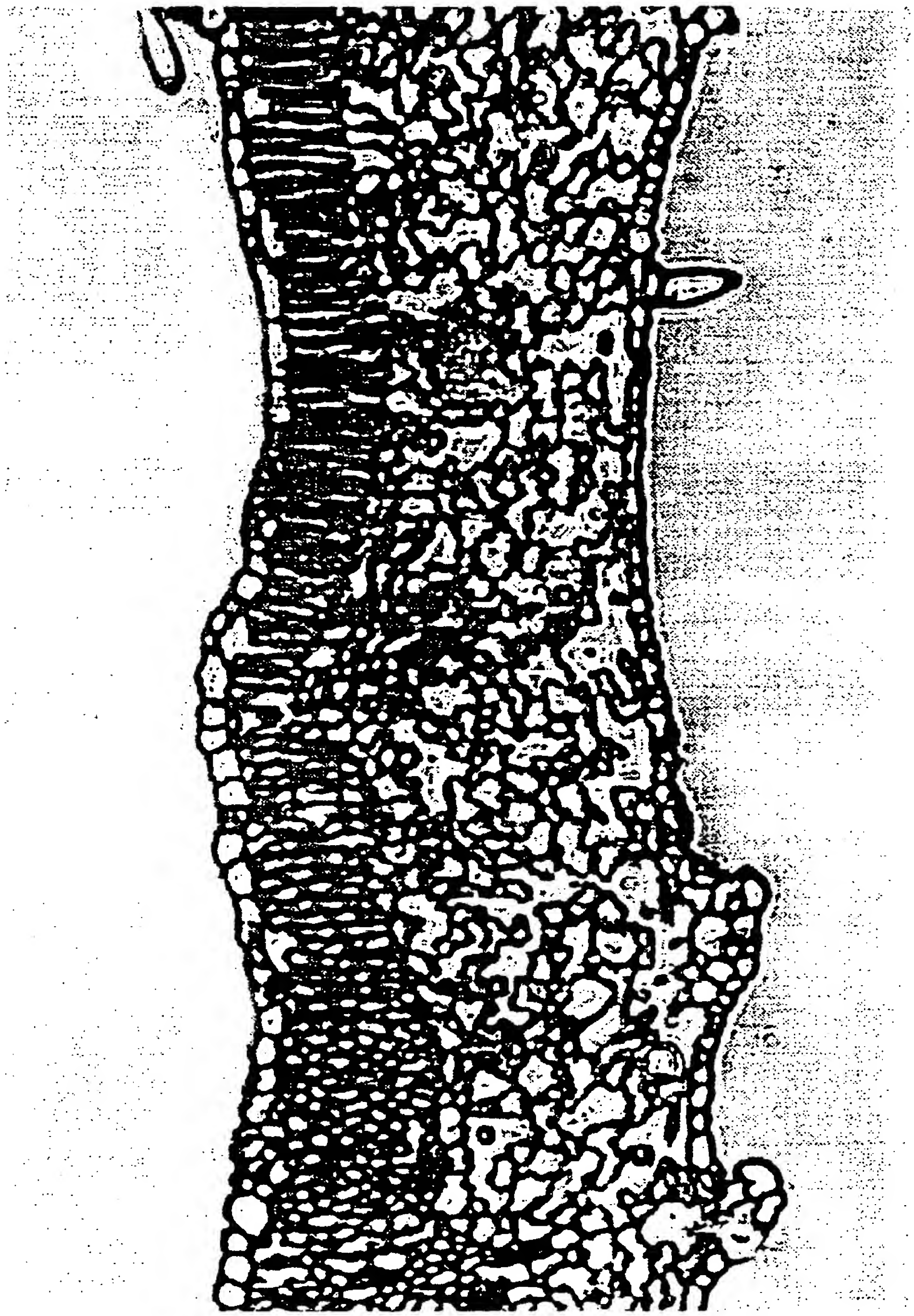

Figure 11

```
   1 CCGCTCATGA TCCCTGAAAG CGACGTTGGA TGTTAACATC TACAAATTGC
  51 CTTTTCTTAT CGACCATGTA CGTAAGCGCT TACGTTTTTG GTGGACCCTT
 101 GAGGAAACTG GTAGCTGTTG TGGGCCTGTG GTCTCAAGAT GGATCATTAA
 151 TTTCCACCTT CACCTACGAT GGGGGGCATC GCACCGGTGA GTAATATTGT
 201 ACGGCTAAGA GCGAATTTGG CCTGTAGACC TCAATTGCGA GCTTTTTAAT
 251 TTCAAACTAT TCGGGCCTAA CTTTTGGTGT GATGATGCTG ACTGGACAAA
 301 TTCAACCCAA TAAGCACATT CCTCTTATAA GATCCATCCC AATAACATGT
 351 AAGTTCAAGG ACTCTAACCA CACACAAATT CACATTTCAT TTGTTAATCA
 401 CCAAAAACAT CTTAAGAATC AACAAAAAGC AAGTAGAATG TATCACTCAC
 451 ATTAACTTGC ACAAAGAAAT TCTTTGGCTC ATAACAACTG CTGATCTTGA
 501 AAAAGGAAGA AAAACAGATA TTTACAAAGA GAGACGAGAA AAGTAGCATT
 551 GTTCATGATT TACCAGCTTT TGTCCCATCA GAATACCTCT GTCATTTCAA
 601 TATTCTTTTG ATTGCTTGGN ACTTGTTCAA TCACATTGTT GCTATCTTTA
 651 ACTGATCTCG ATCCTACTGT TCTTGTATAG CACTGAGTTA GAACCAAAGA
 701 AGCACATCTA AGAACTACAT TTGCACTATT TGCAATTATA GAGCTTAAAT
 751 ATAGCCAGTG TTTTCTGACT AAACGAACGA TTGAGATCAA AAATACAATT
 801 CCACATATAG CACCTGAAAT AAGTAACGGA CCTGAGAACA ACTCTGGTCC
 851 TAATCCAGGA TCATGTTCCA CCAGCCCGGG CCGTCG
```

Figure 12

UQ11 carries a Ds insertion in the RB of the T-DNA

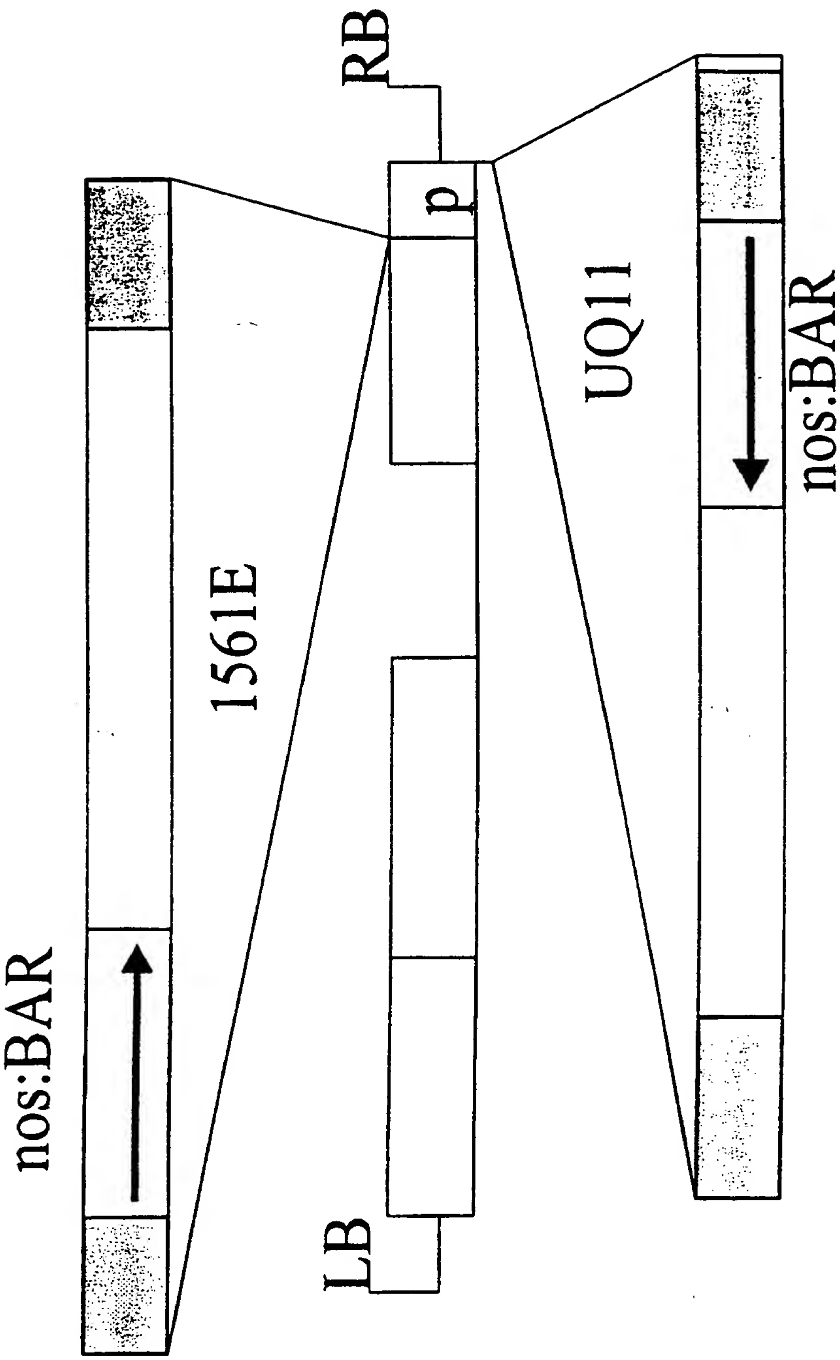

Figure 13